THE MATURATION AND SEGMENTATION OF THE EGGS OF LEPTOPLANA (Spt)

by

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INTRODUCTION

The purpose of the following study of the polyclad, Leptoplana, has been to discover the behavior of the eggs during their maturation, and subsequent segmentations under conditions as nearly normal as possible. The work has been carried on under the direction of Dr. Mary T. Harman. I desire to express my appreciation of the generous assistance and interest Dr. Harman has given me throughout the work. I also wish to thank Dr. J. E. Guberlet, of the University of Washington, who identified the worms.

LITERATURE

The spiral nature of the cleavage of the polyclads up to a late stage of segmentation has been demonstrated by Lang (1884), and Surface (1907), as well as by previous investigators (Girard, 1854, Keferstein, 1868, Goette, 1878, 1882, Selenka, 1881). In this respect the development of the polyclads closely resembles that of molluscan and annelidan eggs (Conklin, 1897, Mead, 1897, and Wilson, 1898). For this reason a detailed study of the behavior of the polyclad eggs in their segmentations

is of interest from a phylogenetic standpoint. However, the difficulty in observing and handling the eggs, due to their opaque nature and their tough capsule or shell, through which fixing agents and stains penetrate with difficulty, seems to have discouraged a most complete embryological investigation except in a few forms.

According to Lang, the earliest investigators of the embryology of the polyclads were Girard (1854), who described the cleavage as total and equal; Vaillant (1866, 1868), who described the segmentation into two, four and eight cells regularly arranged, but said nothing as to the size and appearance of the cells; and Keferstein (1868), who observed the extrusion of the polar bodies and described the spiral nature of the cleavage. These were followed by Halles (1878, 1879), who held that fertilization occurs previous to the time of egg laying, and observed the giving off of the polar bodies, and one quartet of micromeres; Goette (1878, 1882a, 1882b), who also observed one quartet of micromeres; and Selenka (1881), who observed fertilization directly, and also the extrusion of the polar bodies and the formation of two quartets of micromeres.

Pereyaslawyew (1885) reported the first cleavage in <u>Acoela</u> and other forms as equal and succeeding cleavages as unequal and spiral.

Wheeler (1894) described fertilization in <u>Planocera inquilina</u> as "hypodermic impregnation". He also found that the polar bodies were extruded after the eggs were deposited.

Wilson (1894, 1898) described the cleavages as spiral and unequal.

MATERIAL AND METHODS

The material for the study of the eggs of <u>Leptoplana</u> was obtained during the months of June, July and August, 1927, at the Puget Sound Biological Station. Due to the nature of the study, practically all direct observations have been made upon the living material. The worms live in tide pools and deposit their eggs in masses on the under surfaces of small stones. During the period of high tide the pools were not exposed, thus the material could be collected only at definite times. There was no difficulty experienced in keeping the worms and egg masses in the laboratory. The sea water on them was changed once a day. In some cases the worms deposited

eggs while in the laboratory, either upon stones that were kept in the jars or on the sides of the glass vessels. Observations were made on eggs laid in the laboratory and on masses brought in from the tide pools. Usually a part of a mass was removed from the stone for study, and the remainder of the mass returned to its natural habitat to be checked later.

A detailed study was made of the cleavage processes of several eggs within a mass, and later the stage of development was compared with that of eggs from the same mass which had been allowed to continue development in the natural environment. Comparisons were also made with eggs from other masses, and checked with preserved material from each mass studied. The eggs were fixed in Bouin's or Gilson's solutions. Borax carmine was used for staining. It was found necessary, however, to either remove the eggs from the capsules or to tear the capsules into pieces before the stain could penetrate to the eggs. The eggs were then dehydrated, cleared, and mounted in balsam. Serial sections of some of the eggs were made and the sections restained in Delafield's haematoxylin.

NOMENCLATURE

The system of nomenclature followed in this paper is that used by Conklin (1897) in his paper on the embryology of Crepidula. Each of the four quadrants of the egg are designated by the first four letters of the alphabet, A, B, C and D. The quartets of cells separated at various times from the macromeres are designated by small letters and coefficients; thus, the first quartet of micromeres and their derivatives are designated 1a, 1b, 1c, 1d, $1a^1$, $1b^1$, $1a^2$, $1b^2$, etc, the second quartet as 2a, 2b, 2c, and 2d. The term quartet is employed as Kofoid (1894) used it to designate a group of four cells of the same generation, one of which belongs to each of the quadrants of the egg. The four macromeres are the basal quartet, the first group of micromeres separated from these the second quartet, and the second group the second quartet. The animal and vegetative poles are considered the fixed points in the egg. Of the micromeres the stem or parent cell is considered as the upper one (Conklin, 1897). If the division is to the right, that is, if the upper cell lies to the right of the lower when viewed from the animal pole, it is spoken of as dexiotropic or clockwise. If the upper cell lies to the left of the lower it is spoken of as anticlockwise or laeotropic (Lillie, 1895).

OBSERVATIONS

The worms and egg masses were found in abundance during the latter part of June and the early part of July. During the last week of July the tide pools were examined daily, as was the usual custom, but no new egg masses were discovered and the worms had entirely disappeared. Throughout August the worms were plentiful but no new egg masses were found, although the pools and stones were examined regularly.

The Egg Mass

A newly deposited egg mass is light in color in comparison with the older masses which become a dull greenish-brown color. This sometimes makes it difficult to locate the older masses on the stones. The change in color from day to day in a single mass of eggs is definitely noticeable in both the masses kept in the laboratory and those left in the tide pools.

In the laboratory, eggs were always deposited early in the morning and cleavage began a few hours later. Likewise, new egg masses were found in the tide pools only early in the morning, and in many cases cleavage had already commenced when the masses were examined.

The eggs are deposited in crust-like masses on the under surface of stones and are closely cemented to the rock. Each egg is enclosed within an extremely tough but clear capsule or membrane. The eggs are closely but irregularly imbedded together in a single layer, so that the mass can be cut from the stone without injuring the eggs. This made it possible to watch the cleavage processes under the microscope. The main difficulty experienced in observing the cleavages of the living egg was the opacity. This was due to the amount of yolk in the egg.

The individual egg is comparatively large, spherical in shape, lies to one side of the center of the capsule, and is made up of a uniformly dense mass of granules, fig. 1. The newly laid egg is opaque, and its surface is not always smoothly round. A more or less granular substance surrounds the egg within the shell.

Cleavage occurred in a normal manner in all the masses which were laid in the laboratory except one. This one had an unusual appearance when it was deposited, and it degenerated within a few days.

Maturation

Fertilization of the egg has not been observed. Two maturation divisions occur after the egg is deposited,

and two very small polar bodies are extruded and remain in contact with the egg for a while, figs. 1 and 2. The second polar body is given off soon after the first. After these polar bodies are cast off there is a noticeable clearing of the cell so that the subsequent cleavages can be easily observed. In only one case was the approximate time between the deposition of the eggs and the extrusion of the polar bodies secured, and in this, the worm was observed crawling off from a newly laid mass of eggs. Examination showed that the eggs were one-celled and opaque. Thirty minutes later, several eggs cast off the first polar body, and within a little while became much less opaque. Within two hours after the first maturation most of the cells in the mass were segmenting.

Cleavage

The cleavages in Leptoplana occur in intervals of approximately two and one-half to three hours. About twenty minutes elapse from the time a cleavage furrow is first visible until the daughter cells are completely separated. The blastomeres rotate at the end of the segmentation, after which there is a quiescent period until the beginning of the next segmentation. Variations in

the duration of these intervals were noted which may have been due to environmental conditions. In each case in which artificial light was used for observation, thus causing an increase in the temperature, the segmentations occurred more slowly. Furthermore, development normally takes place on the under surfaces of stones, a condition in which light is largely excluded. In comparing the stage of development of those eggs which had been under observation with natural light with the remainder of the mass which had been left in the normal environment, it was found that the two were in approximately the same stage of development. Thus, temperature appeared to have a greater effect upon the eggs in their cleavages than light.

The First Cleavage. The first cleavage is polar, total, and results in two slightly unequal blastomeres, fig. 6. The egg elongates at the beginning of the segmentation. The cleavage furrow may appear first at one side of the egg, but more often it appears at both sides at the same time. The two blastomeres are at first nearly spherical, and touch each other by only a comparatively small surface, figs. 5 and 6. Later the cells are drawn toward each other, especially at the animal pole,

and the surfaces of contact become much longer and flattened, fig. 7. The greater part of the yolk collects at the vegetative pole, which gives it a more dense appearance.

The Second Cleavage. The second cleavage is polar and perpendicular to the first. The cells resulting from this cleavage are also slightly unequal. The larger blastomere, CD, divides in advance of the other, fig. 8, often resulting in a temporary three-celled stage, figs. 9 and 10. This cleavage really consists of two independent furrows, one of them appearing earlier than the other. At the end of the cleavage the cells shift so that the two smaller cells, B and D, lie at a higher level and tend to come in contact with each other at the animal pole, forming the so-called animal polar furrow, fig. 11. The two larger blastomeres, A and C, come in contact at the vegetative pole, forming the vegetative polar furrow, fig. 12.

The Third Cleavage. At the end of the resting period the blastomeres begin to shift and lighter places appear near the centers of cells A, C, and D, fig. 13. In a very short time these cells begin to show cleavage furrows in the equatorial plane, fig. 14. The divisions are not synchronous, as cell D begins to divide first; next cells A and C begin to bud off micromeres at almost the

same time; and lastly, cell 1 begins to divide, figs. 14 to 17. The divisions result in two quartets of cells of decidedly unequal size, fig. 18. The basal quartet which contains the bulk of the yolk material, becomes the macromeres, A, B, C and D, and the apical quartet becomes the micromeres, 1a, 1b, 1c, and 1d. There is also a strongly dextrotropic rotation of the micromeres during and at the close of the cleavage, as shown in figures 14 to 19, until they finally come to lie in the furrows between the macromeres. Cell 1a lies between A and B, 1b lies between B and C, 1c lies between C and D, and 1d lies between D and A, figs. 19 and 20.

The Fourth Cleavage. At the beginning of the fourth cleavage there is a further elongation of the micromeres, fig. 21, until they come to lie almost directly over their corresponding macromeres. At this stage the micromeres become exceedingly transparent, so that the egg might almost be taken for a four-celled stage when viewed from the animal pole. At the time that the micromeres begin to shift, the macromeres appear more dense near the center. Then, very quickly and simultaneously, a second quartet of micromeres, 2a, 2b, 2c, and 2d are separated from A, B, C and D. This second quartet is slightly larger than the

first quartet. The movement of this second quartet of micromeres is strongly laeotropic, so that 2a finally comes to lie in the furrow between A and D, 2b between A and B, 2c between B and C, and 2d between C and D, fig. 24.

At the same time that the macromeres are dividing, cleavage furrows appear in the first quartet of micromeres, fig. 22, and soon after the cells are completely separated, resulting in eight cells of almost equal size, fig. 24. The stem cells $1a^1$, $1b^1$, $1c^1$ and $1d^1$ shift dexiotropically, and $1a^2$, $1b^2$, $1c^2$ and $1d^2$ shift laeotropically. When the egg has passed into the resting stage the whole is very compact, with the twelve micromeres fitting closely into the furrows between their adjacent cells, fig. 24.

DISCUSSION

As has been previously stated the eggs of Leptoplana are made up of a uniformly dense mass of granules, and there is no differentiation into a more dense inner portion and a clearer outer portion as has been found by Selenka, Goette, Hallez, and Lang (Lang, 1884) in some polyclad eggs. Surface (1907) found the eggs of Planocera inquilina and Lang (1884) the eggs of Discocoelis tigrina

to be of uniform density throughout, as are the eggs of Leptoplana. In Leptoplana never more than one egg was observed within a capsule, but Surface found that in P. inquilina two eggs are sometimes deposited in a single membrane, each of which develops into a normal embryo.

It is of interest to note that in Leptoplana the newly laid egg masses were found during only a part of the summer. This fact tends to indicate that there might be definite reproductive periods in this particular group of Turbellaria.

Opinions differ as to when fertilization takes place. Lang (1884) holds that copulation occurs before egg laying but that fertilization may not have occurred by the time of the deposition of the eggs. Furthermore, according to Lang (1884), Balles was of the opinion that fertilisation occurs previous to egg laying. Lang further states that Selenka, who was able to observe fertilization in the polyclads directly, was of the opinion that the spermatozoon enters the egg and lies there until after the polar bodies are extruded. Wheeler (1894) makes this statement in regard to fertilization in Planocera inquilina: "There is undoubtedly in this species a true 'hypodermic impregnation', to use Professor Whitman's

term. In the aquarium the sexually mature animals crawl over one another and thrust their stylet shaped penes into one anothers' bodies at any point."

Although fertilization may occur previous to, or as the eggs are being deposited, the maturation divisions in Leptoplana do not occur until after deposition. Apparently these divisions take place soon after the egg is laid, and there is a resting period before segmentation begins. The polar bodies, which are extremely small, remain attached to the egg for a while. In other forms, Planocera inquilina (Surface, 1907), Thysanozoon (Selenka, 1881) and Discocelis tigrina (Lang, 1884) they do not remain attached. Surface also found that the eggs of P. inquilina went through some remarkable contortions during maturation, a condition that was not observed in Leptoplana.

Considerable variation in the behavior of the different forms which have been studied is evident, although they are all essentially alike. As was mentioned above, the cleavage is spiral. It is also total and unequal, and the blastomeres do not always divide simultaneously. The intervals of time between the successive cleavages also vary in different forms. In P. inquilina, in which the cleavages occur about every hour, the intervals are

relatively short, in comparison with Leptoplana in which the cycle is from two and a half to three hours in length. The slower rate seems to be more constant in polyclads (Lang, 1884).

The inequality in the size of the blastomeres from the first cleavage is an outstanding characteristic of these eggs. According to Lang (1884) and Surface (1907) the difference in size of the first two blastomeres is very constant in polyclads. Lang says "Ich habe diese allerdings wenig auffallende Verschiedenheit in der Grösse der zwei ersten Blastomeren, die Selenka bei Tyrsenozoon und Eurylepta constatirte nicht nur bei Discocoelis tigrina, sondern auch bei allen Pseudoceriden und Eurylepta nachweisen können. Ich glaube dass sie auch bei allen Leptoplaniden existirt, obschon sie hier schwer nachweisbar ist." Girard (1854) described the cleavage of Planocera elliptica as total and equal throughout. Later investigations on the polyclads have shown the cleavages to be unequal in those forms studied.

In Leptoplana the cells resulting from the second cleavage are also unequal in size, although this inequality is not as marked as in D. tigrina and P. inquilina. This third cleavage results in two quartets of cells which

are decidedly unequal in size, a condition similar to that in D. tigrina. In F. inquilina the difference in size, while sufficient to be easily recognized, is not as great as in Leptoplana.

There is a constant rhythm in the segmentation of the eggs of Leptoplana. In the second cleavage, the larger cell begins to divide in advance of the other. Lang found that in the case of Discocelis the larger divides first, as: "Die Theilungerfolgt aber nicht ganz gleichzeitig, die grösseren Furchungskugel theilt sich vielmehr etwas früher als die kleinere." In the third cleavage the larger cells also begin to divide in advance of the smaller. In the fourth cleavage it is a notable and constant fact that the macromeres split off a second quartet of micromeres before the first quartet completes its division. It is usually thought that in the case of unequal holoblastic segmentation that the presence of yolk material tends to retard division in the larger cells, so that the cells containing less yolk divide more rapidly, (this is the condition in the frog's egg) but in the case of the polyclads the presence of yolk material apparently does not retard cleavage. The cleavage of gasteropod eggs is like that of the polyclads in that the cells containing yolk divide in advance of the others.

Conclusions

The following conclusions regarding the maturation and segmentation of the living eggs of Leptoplana may be made:

1. The maturation divisions take place after the egg is deposited, and the second divisions follows closely after the first.

2. The cleavages of the egg are holoblastic and unequal.

3. The blastomeres do not divide synchronously.

4. The presence of yolk material in the cells does not retard cleavage since the larger cells divide in advance of the smaller.

5. The division is spiral and the cells rotate in dexiotropic or laeotropic directions.

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EXPLANATION OF PLATES

Plate I

Fig. 1. Newly laid egg. sh, shell.

Fig. 2. First polar body. 1 p.b., first polar body; sh, shell.

Fig. 3. Egg at close of maturation divisions. 1 p. b., first polar body; 2 p. b., second polar body.

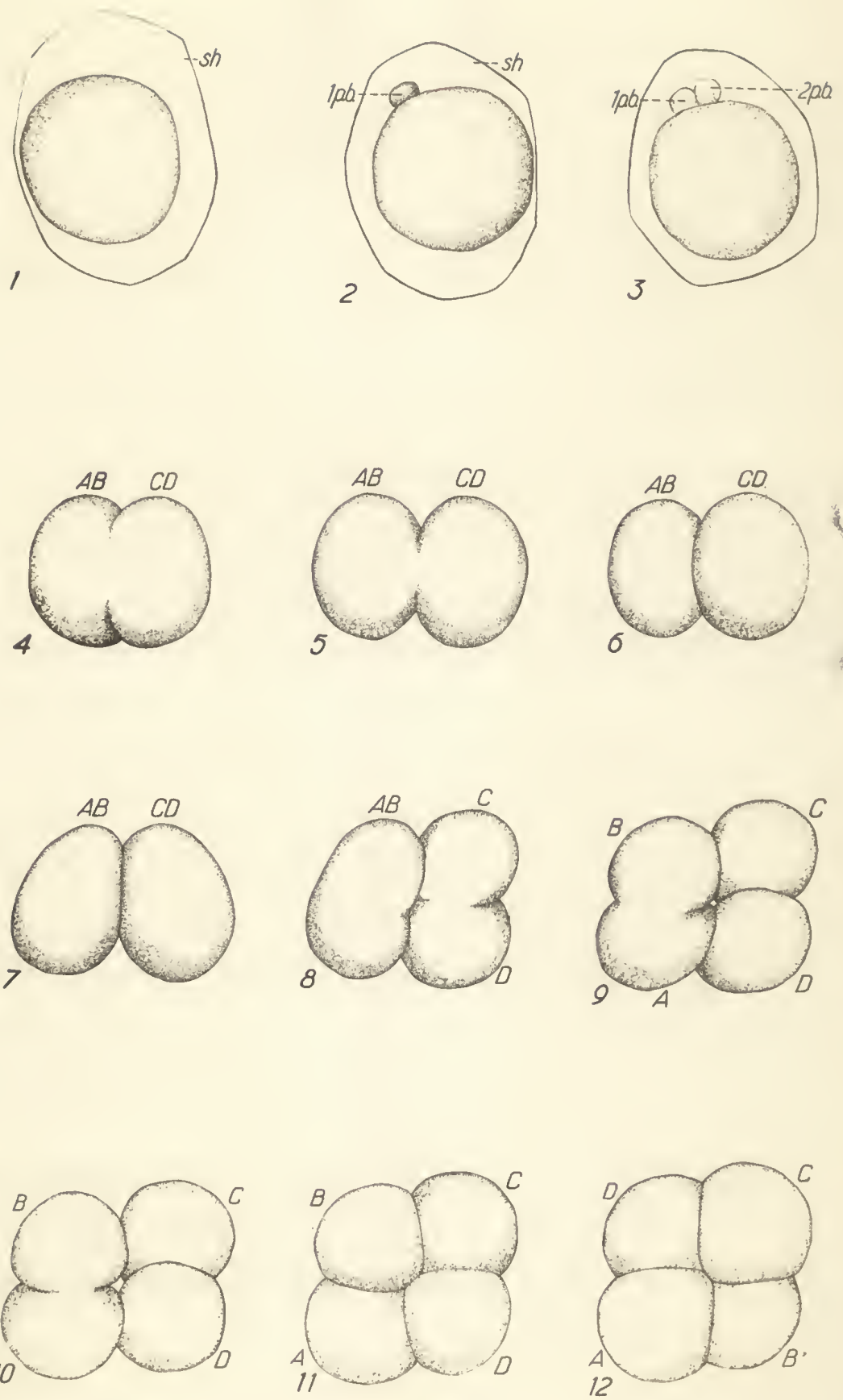
Figs. 4, 5 and 6. Stages in first cleavage. AB, larger cell; CD, smaller cell.

Fig. 7. Resting stage after first cleavage, showing flattening of blastomeres against each other. AB, smaller cell; CD, larger cell.

Figs. 8, 9 and 10. Stages in second cleavage. CD divides in advance of AB.

Fig. 11. Resting stage at end of second cleavage, from animal pole. B and D, the smaller cells, in contact with each other at the animal pole.

Fig. 12. Same egg as in fig. 11, from vegetative pole. A and C, the larger cells, in contact with each other at the vegetative pole.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12

Plate II

Figs. 13, 14, 15, 16 and 17. Stages in third cleavage. A, B, C and D, macromeres; 1a, 1b, 1c and 1d, first quartet of micromeres.

Fig. 18. End of third cleavage. A, B, C and D, macromeres; 1a, 1b, 1c and 1d, first quartet of micromeres, shifting dexiotropically.

Fig. 19. Resting stage following third cleavage, lateral view. Labelling as in fig. 18.

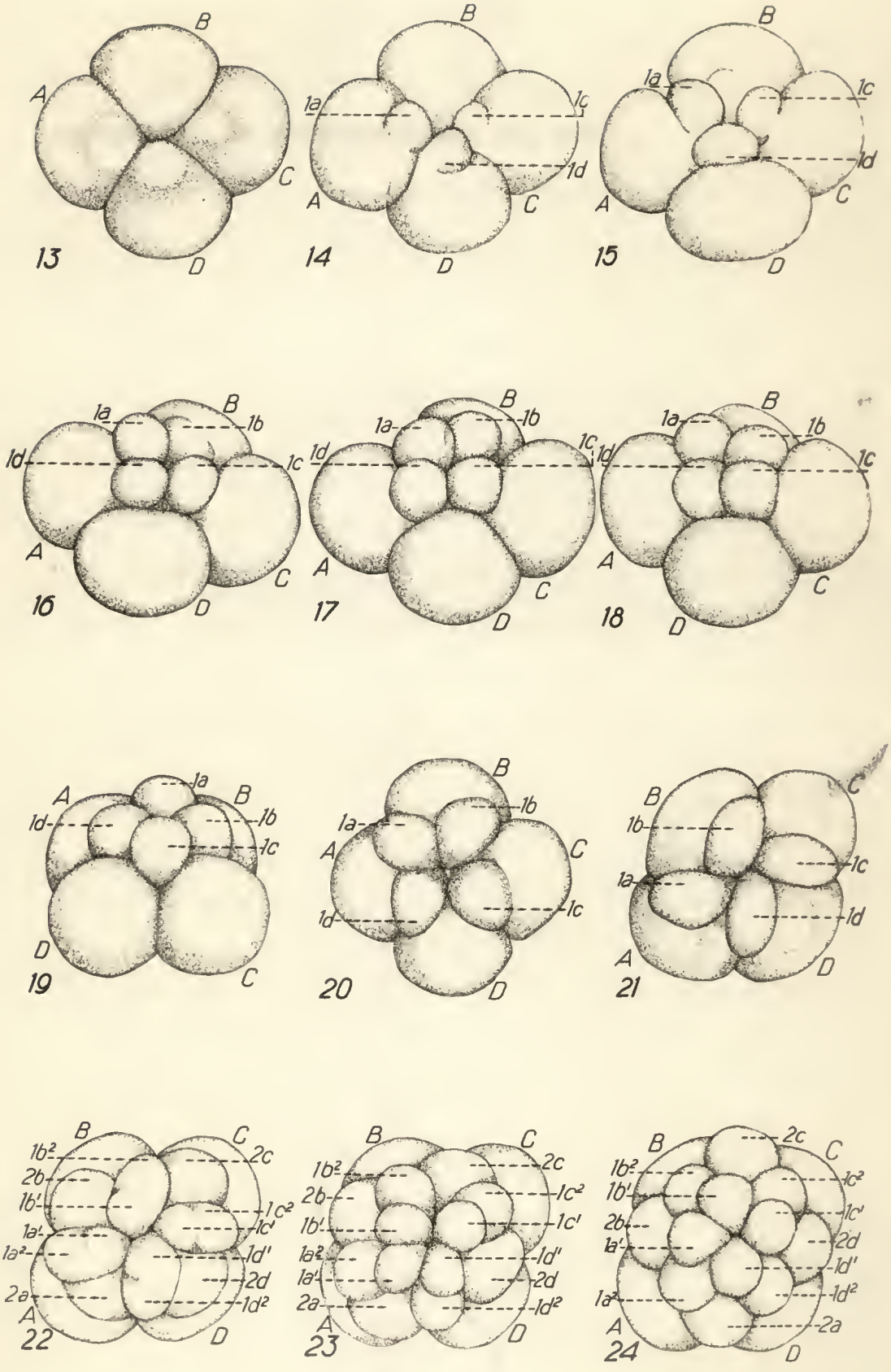
Fig. 20. Resting stage following third cleavage, from animal pole. Labelling as in fig. 18.

Fig. 21. Beginning of fourth cleavage. Elongation and shifting of 1a, 1b, 1c and 1d. Labelling as in fig. 18.

Fig. 22. Completion of second quartet of micromeres. 2a, 2b, 2c and 2d from A, B, C and D. Appearance of cleavage furrows in 1a, 1b, 1c and 1d, first quartet of micromeres. A, B, C and D, macromeres.

Fig. 23. Completion of division of 1a, 1b, 1c and 1d, first quartet of micromeres. Cells $1a^1$ and $1a^2$ from 1a, $1b^1$ and $1b^2$ from 1b, $1c^1$ and $1c^2$ from 1c, and $1d^1$ and $1d^2$ from 1d. First quartet of micromeres, $1a^1$, $1b^1$, $1c^1$ and $1d^1$, shifting dexiotropically; $1a^2$, $1b^2$, $1c^2$ and $1d^2$, micromeres separated from first quartet, shifting laeotropically; 2a, 2b, 2c and 2d, second quartet of micromeres, shifting laeotropically. A, B, C and D, macromeres.

Fig. 24. Resting stage at end of fourth cleavage, from animal pole. Labelling as in fig. 23.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